Anderson, R. J.

✓ Copy to Mr. White

December 28, 1937.

My dear Doctor Anderson:

You remember that I reported at the Committee meeting that there was a greater cellular reaction to the phosphatide from A-14 than to that from H-37 -- really very considerably greater. Indeed, we found a somewhat larger reaction in each of the new phosphatides than to the material from H-37.

On this account we decided to restudy these phosphatides with acid-fast stains, with the result that there are certainly some tubercle bacilli in the phosphatides from A-14 and from A-10. Concerning the others, our tests are not yet complete.

To apply the acid-fast stain to the phosphatide is really a matter of very considerable difficulty because the material does not stick to the slide; with the wax it is quite different, - the material sticks well and is, of course, strongly acid-fast.

The technique, as you know, is first to stain in a solution of fuchsin in water, heating the stain on the slide until it steams. The phosphatide appears to stick very well through this part of the process. Then the stain is drained off and the slide decolorized in 3% nitric acid made up in 95% alcohol. During this process most of the phosphatide disappears. Viewed under polarized light, only a few particles of doubly refractile material are left on the slide. When the slide is examined under oil immersion lenses, there are a few masses of granular material, not acid-fast, but in the case of the phosphatide from A-10 and A-14, some of these contain unequivocal small clumps of acid-fast rods, undoubtedly tubercle bacilli. We have not yet put these little masses under polarized light so we do not know whether the material in which the bacilli are imbedded is doubly refractile and therefore probably phosphatide or not.

I should like to ask you whether the treatment with 95% alcohol and nitric acid is likely to degrade the phosphatide to some simpler state or not. Since so much of the phosphatide disappears from the slide, it is not possible to tell how many bacilli were actually present in the original material. All we can say is that there certainly were some.

You will remember that we had felt pretty sure that there were no bacilli in the phosphatide from H-37 which you prepared in 1932. Considering the difficulties of the technique which we have just described, it is perhaps possible that we can never say that not a single bacillus has passed the filter. But we can make the milder statement that we cannot find any. We took some of the 1932 material and dissolved it in the mixture of 7 parts of ether and 3 parts of chloroform which Doctor Joyner had found out allowed one to centrifuge tubercle bacilli out of material. After dissolving some of the phosphatide in this mixture and submitting it to prolonged centrifuging, a residue was taken up and stained for tubercle bacilli; we found none. Also, the guinea pigs which had received six intradermal injections of this phosphatide did not become sensitized to tuberculoprotein. which brings a little biological evidence to bear on the point and probably means that there certainly could not have been any great numbers of tubercle bacilli in the material. We certainly know that when a small amount of tuberculoprotein is added to the phosphatide and injected intradermally, the guinea pigs become sensitized even when only small amounts of the tuberculoprotein are used.

In one preparation of the 1932 phosphatide I found one red bacillus in a mass of material which had not been decolorized. This is a condition which would not be accepted as proving tubercle bacilli in sputum, but it is an observation which makes me say that perhaps a 100% elimination of bacilli is a hard criterion to set up.

Have you any more of the A-14 phosphatide, and if so, do you not think it would be a good plan to refilter it and let us have some of it both for the cellular reaction and, if you have enough, for the sensitizing experiments? It would take about 30 mgms. for the cellular studies and approximately 100 mgm. for the sensitizing experiments.

We are working both on the polysaccharides and on the whole series from the defatted bacilli and will let you have our findings as soon as possible.

I am glad you enjoyed the Committee Meeting. I certainly did too and think that Doctor Boissevain had certainly calmed down a good deal.

Cordially yours,

Florence R. Sabin.

Doctor R. J. Anderson,
Sterling Chemistry Laboratory,
225 Prospect Street,
New Haven, Connecticut.